MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD-MAPPING

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I. Introduction

Colon tracing and microstimulation experiments which were initially started in previous quarters continued during this quarter. The tracing studies used the transynaptic tracer pseudorabies virus (PRV) to examine the location and distribution of PRV labeled neurons in the central nervous system of the cat following injection of PRV into the colon. During this quarter these studies were extended to determine brainstem and afferent labeling following PRV injections into the distal and mid colon. By allowing the animal to survive for 110 - 120 hours following colon injections, supraspinal labeling was seen in the dorsolateral tegmentum at the level of the pons in an area which includes the locus caeruleus, subcaeruleus, and the pontine reticular formation. In addition the afferent neurons in the dorsal root ganglia were labeled in these animals and a figure showing the afferent distribution is presented below (see Figure 4).

Microstimulation studies also continued during this quarter. In the most recent experiment three saline filled balloon catheters were used to monitor pressure changes in the proximal and distal colon as well as in the region of the external and internal anal sphincters (see Figure 5). Spinal cord and ventral roots were electrically stimulated to determine stimulus parameters and sites which produced the maximal responses. The details of these experiments are presented below.

II. Tracing Studies Using Pseudorabies Virus (PRV) to Label Colon Neurons and Interneurons at Spinal and Supraspinal Sites.

Previous studies from this laboratory have shown that PRV injected into the mid and distal colon labels neurons and interneurons at various sites in the lumbosacral spinal cord (see

previous progress reports and Figures 2 & 3, this report). In experiments conducted during this quarter PRV was injected into the mid/distal colon of the cat and the survival time was extended to 120 hours in order to allow time for transport to supraspinal sites. These experiments were conducted in a similar manner as described in previous progress reports. Figure 1 depicts some of the spinal circuits and methods used in these studies.

The mid and distal colon was injected with PRV at 50 to 60 sites (5µl at each site) along its length. The distal most 6 cm of the colon, measured from the anal opening, and the proximal colon (about 10 cm) were not injected. (These sites will be injected in separate tracing experiments which will examine the internal and external anal sphincter and the proximal colon.) To allow transport to supraspinal sites the survival time was increased from our normal time of 60 - 92 hours to 120 hours. The tissue was processed using an antibody to the virus and otherwise standard techniques described in detail in previous progress reports. The majority of supraspinal labeling in these experiments was seen in the brainstem at the level of the pons. Figure 2 (right side) shows the type of labeling seen at this pontine site. Neurons are labeled in the dorsolateral pontine tegmentum including: the locus caeruleus, subcaeruleus, periaquiductal grey, pontine reticular formation, and the parabrachial nuclei. The majority of labeling seen was at this site and extended rostrocaudally only a few mm. Labeling at these sites were somewhat surprising since bladder and penile injections of PRV in the cat label these same sites. This brainstem site was once thought to be involved exclusively in micturition and termed the pontine micturition center. It now however, appears to be an autonomic center which provides descending input to sites in the lumbosacral spinal cord and very likely modulates bladder, colon and sexual function. It is still unknown whether the same or different neurons project to

interneurons which control the activity of each specific organ.

With extended survival time in these experiments primary afferent neurons in the dorsal root ganglia are also labeled. Figure 4 shows the distribution of colon primary afferent neurons at various levels of the spinal dorsal root ganglia (DRG). The S2 DRGs provides a major afferent input to the spinal cord although dorsal root ganglia at other levels also provide significant input to the spinal cord neurons. The S2 spinal cord segment also provides a major portion of the excitatory outflow to the distal colon, suggesting that these neurons are positioned to play a major role in reflex control of colon motility.

These types of tracing experiments will continue during the next quarter where sphincter and proximal colon will be examined with PRV tracing experiments.

III. The Effects of Spinal Cord Microstimulation on Colon Motility - Location and Stimulus Parameters

These experiments are a continuation of studies begun in previous quarters. The major difference in these studies have been the use of three balloons at various levels of the colon to record pressure changes to ventral root and spinal cord microstimulation. The methods used in these experiments have been described in detail in previous progress reports and are summarized schematically in Figure 5. The use of three balloons connected to pressure transducers allows recording of colon pressure changes along its entire length and also allows for monitoring changes in internal and external (IAS & EAS) pressure. The major drawback in using several balloons to monitor pressure is that each additional balloon produces stretching of the colon at the time of surgery and during positioning of the balloons. Stretching the colon often produces a

long lasting (several hours) inhibition of colonic activity. The inhibition can be so intense as to block the excitatory input from ventral root stimulation. This inhibition is mainly seen with colon smooth muscle and not the striated muscle of the EAS. In some experiments, in order to proceed with mapping studies, small doses (3 mg/kg) of the β adrenergic antagonist, propranolol was used to block the inhibition seen following surgery. Sectioning of the sympathetic inhibitory nerves (hypogastric and lumbar colonic nerves) also proved useful in some experiments.

In all our mapping experiments the sacral ventral roots are first stimulated with a hook electrode to determine the segments which provides the major excitatory outflow to the colon. Figure 6 shows a typical response for a single animal where S2 provides major outflow to the colon smooth muscle and S1 provides the major input to the sphincter, although S2 also provides some sphincter activity. S3 in many animals often provides significant excitatory input to the colon. Figure 7 is the mean response recorded at three colon sites for 5 to 8 animals (three balloons were used in only five animals), to S1, S2, or S3 ventral root stimulation. The largest sphincter responses are from S1 and S2 with almost no response from S3 (Figure 7, top row). Pressure changes in distal colon are seen with all three sacral ventral roots with the largest mean response from S2 stimulation (Figure 7, middle row). The pressure changes from proximal colon were in general weaker and more variable than those from distal colon. S1 rarely produces any response, while S2 and S3 produces small responses which were quite variable (Figure 7, bottom row).

The ventral root (usually S2) which produced the largest response of the distal colon was assumed to originate from the spinal segment with the greatest number of excitatory neurons. This segment (usually S2) was mapped first in our microstimulation experiments. Figure 8 show

a typical response from S2 spinal cord microstimulation at a depth of 1.2 mM from the spinal cord surface at the dorsal root entry zone (DREZ). The proximal colon response was usually very small, while the sphincter and distal colon responses larger. The preferred frequency for maximal colon contraction was typically between 10 and 20 Hz. Figure 9, 10, and 11 shows the mean effects of changes in various stimulus parameters for sphincter, distal colon, and proximal colon. Sphincter and distal colon had the lowest threshold for activation with a mean between 20 and 40 μA while proximal colon had slightly higher with a threshold between 40 and 60 μA (Figure 9). The preferred frequency of stimulation was 15 Hz, although proximal colon showed high variability (Figure 10). It should be remembered that sphincter pressure being recorded in this experimental setup is a combination of smooth muscle of the IAS and striated muscle of EAS. The smooth muscle of the colon and sphincter seem to prefer a low (15 Hz) frequency of stimulation while striated muscle typically responds best at somewhat higher (30 - 40 Hz) frequency of stimulation. This data would suggest that the smooth muscle of the IAS is probably dominating much of the sphincter response.

The difference in preferred frequency between the smooth and striated muscle is in part due to the neurons in the peripheral ganglia of the autonomic nervous system which do not transmit high frequency input very efficiently.

The longer pulse widths seem to be more effective when stimulating the spinal cord as compared to ventral roots which prefer short pulses (0.05 to 0.1 msec). This is probably due to the fact that microstimulation of the spinal cord activates cell bodies and axons while root stimulation activates only axons. Several published studies have concluded that neuronal fibers or axons are best activated by short pulses while cell bodies prefer significantly longer pulse

durations. Our histological results would suggest that our microelectrode tips are in grey matter where a mixture of cell bodies and axons are most likely stimulated with our electrodes. The stimulus parameters used in all our mapping studies are typically 100 μA, 15 Hz, and 0.2 msec. Although 0.2 msec is not the best pulse width (Figure 11), 0.2 msec is used to reduce the chances of tissue damage from high current density.

The location of sites within the S2 spinal cord which produces the largest distal colon responses are shown in Figure 12 (tracts 1 and 2). These sites are typically 1.0 to 1.6 mm and 2.0 to 2.6 mm from the dorsal surface of the spinal cord with a small gap between these areas. In some instances, sites at the very surface of the cord especially at the DREZ produce large responses often accompanied by somatic motor responses of the tail and hindlimbs. These are likely reflex responses due to excessive primary afferent stimulation (Figure 12, tract 2). There are additional sites deep in the spinal cord which produce large non-specific responses of the somatic motor musculature. These are shown in deep, very medial tracts 5 and 6 in Figure 12.

These studies will continue during the next quarter with additional mapping of both colon and sphincter responses in the S1 segment of the sacral spinal cord.

Figure 1. Diagrammatic representation of the extrinsic innervation of the large intestines of the cat. Preganglionic fibers are indicated by solid lines and postganglionic fibers by dashed lines. (+) and (-) indicate respectively the excitatory and inhibitory actions of these nerves. Origin of the nerves at different levels of the neuraxis is indicated on the right side of the diagram. Parasympathetic excitatory pathways originate in the medulla (vagus nerve) or the sacral cord (pelvic nerve). Sympathetic inhibitory pathways arise in the thoracic cord (splanchnic nerve) or lumbar cord (lumber colonic nerves). The lumber sympathetic outflow (hypogastric nerve) also provides an excitatory input to the smooth muscle of the internal anal sphincter (IAS). The striated muscle of the external anal sphincter (EAS) receives an excitatory input form the sacral cord via the pudendal nerves. Pseudorabies virus (PRV) injections were made into distal and middle colon.

Figure 2. **Left side:** Camera lucida drawings at three levels of the sacral spinal cord (S1r, S2r, and S3r; r = rostral) showing the location and distribution of pseudorabies virus (PRV) labeled neurons in transverse sections following injection of PRV into the mid and distal colon. Notice the labeled neurons in the sacral parasympathetic nucleus of S2r and S3r and around the central canal. Labeled neurons from eight transverse sections are superimposed for each drawing of the sacral segments. Bar = 50μ. **Right side:** Camera lucida drawings of the brainstem at the level of the pons showing the location and distribution of PRV labeled neurons in the dorsolateral pontine tegmentum. Notice the labeled neurons just medial to the superior cerebellar peduncle in the locus caeruleus and subcaeruleus. Labeled neurons were often seen extending dorsal into the periaquiductal grey and ventral into the pontine reticular formation. Labeled neurons from 18 transverse sections are superimposed for this drawing. Bar = 900μ.

Figure 3. Camera lucida drawings at three levels of the lumbar spinal cord (L2r, L3r, and L4r; r = rostral) showing the location and distribution of pseudorabies virus (PRV) labeled neurons in transverse sections following injection of PRV into the mid and distal colon. Notice the labeled neurons in the intermediolateral grey (IML) of L2r, L3r, and L4r and around the central canal. Labeled neurons from six transverse sections are superimposed for each drawing of the lumbar segments. Bar = 50μ.

Figure 4. Histogram showing the distribution of labeled afferent neurons in the lumbosacral dorsal root ganglia following injection of PRV into the mid and distal colon. The major sensory input from the distal colon to the spinal cord is at the level of the sacral cord especially the S2 segment. Other lumbar and sacral segments however also provide significant numbers of sensory innervation of the colon. Counts are from left DRG of one animal.

Figure 5. Diagrammatic representation of the experimental setup and methods used in these studies. Three saline filled balloons are positioned at three levels of the colon: proximal colon, mid-distal colon, and at the level of the internal and external anal sphincter. The balloons are connected to three pressure transducers (T) via polyethylene tubing. The outputs from transducers are amplified, recorded on tape, displayed on a chart recorder, digitized and stored in a computer for on-line and off-line display and analysis. The lumbosacral spinal cord is exposed via a laminectomy. Each sacral ventral root is stimulated with a hook electrode to identify the segment (usually S2)

which has the largest exciting outflow. This segment is then probed with fine tipped (400 μ^2 exposed tip) activated iridium electrode. Each 200 μ along vertical tracks are stimulated and the colon and sphincter responses recorded.

Figure 6. Chart recorder output showing intraluminal colon pressure changes at three levels of the colon (proximal, distal and sphincter) to stimulation of three different sacral ventral roots (S1, S2 and S3). Notice that S1 ventral root stimulation produces a large sphincter contraction with little or no colon contraction, while S2 produces large colon responses with small sphincter contraction. S3 produces only small responses. Stimulus parameters: 10.0 V, 0.05 msec, 15 Hz, 30 seconds on and 120 seconds off.

Figure 7. Graph showing the effects of increasing intensity of stimulation of the S1, S2, and S3 ventral roots (columns) on the area under the colon pressure response for each of three balloons located at different sites along the colon (sphincter, distal, and proximal - rows). The first column indicates the effects of S1 ventral root stimulation at increasing intensities for sphincter (top panel), distal colon (middle panel) and proximal colon (bottom panel). The middle column is for S2 and right most column S3. Notice that S1 ventral root stimulation (column 1) produces a large sphincter and distal colon response but not proximal colon pressure change. S2 stimulation (second or middle column) produces a good sphincter response, the largest distal colon pressure change and a large but quite variable proximal colon response. S3 ventral root stimulation (column 3) generates almost no sphincter and only small distal and proximal colon pressure changes. Data points are mean $\pm$ SE for 5 to 7 animals.

Figure 8. Chart recorder output showing sphincter and colon intraluminal pressure changes to microstimulation of S2 spinal cord at various frequencies from 5 to 40 Hz. The depth of the microelectrode tip is 1.2 mM from the dorsal surface of the spinal cord just at the dorsal root entry zone. Notice that preferred frequency of microstimulation is 10 to 20 Hz with a marked drop in amplitude at both lower and higher frequencies. Stimulus parameters: 100 μA, 0.2 msec, 15 Hz.

Figure 9. Graphs showing the changes in sphincter and colon intraluminal pressure to increasing intensity of microstimulation of the S2 spinal cord. Data is mean$\pm$SE percent of the area under the colon and sphincter pressure curves normalized to the largest response for each animal. Notice that the threshold for sphincter and distal colon is 20 to 40 μA, while that for proximal colon is slightly higher 40 to 60 μA. Notice also the responses increase with increasing intensity of stimulation. Stimulus parameters: 10-100 μA, 0.2 msec, 15 Hz, 30 seconds on and 120 seconds off. Depth in the S2 spinal cord varied from 1.0 to 2.4 mM.

Figure 10. Graphs showing the changes in sphincter and colon intraluminal pressure to increasing frequency of microstimulation of the S2 spinal cord. Data is present as in Fig.7. Notice that the best frequency for stimulation is between 10 and 20 Hz with the peak at 15 Hz, although the peak is not as clear with proximal colon response. Stimulus parameters: 100 μA, 0.2 msec, 5 to 35 Hz, 30 seconds on and 120 seconds off.

Figure 11. Graphs showing the changes in sphincter and colon intraluminal pressure to changes in stimulus pulse width, during microstimulation of the S2 spinal cord. Data is present as in Fig.7 and Fig.8. Notice that as pulse width is increasing the response is also increased. Stimulus parameters: 100 μA, 15 Hz, 0.05 to 0.5 msec pulse duration, 30 seconds on and 120 seconds off.

Figure 12. Graphs of colon responses to focal microstimulation at various depths from the dorsal surface of the S3 spinal cord. Responses along four electrode tracts (1,2,5 & 6) are shown at 200 μ increments. The trajectory of the tracts in S3 spinal cord is illustrated by the spinal cord figurine at the center of the figure. Response along tracts 3 & 4 are not shown. Area, peak, and duration are shown for proximal (p) colon recording and distal (d) responses. Stimulus parameters are: 100 μA, 0.2 msec pulse duration, 15 Hz, 30 sec on 120 sec off. Responses at 2.8 mM or below are near or outside the edge of the spinal cord and represent non-specific activation of colon as well as somatic responses. Specific colon responses are best illustrated between 1.0 and 2.2 mM in tracts 1, and 2.

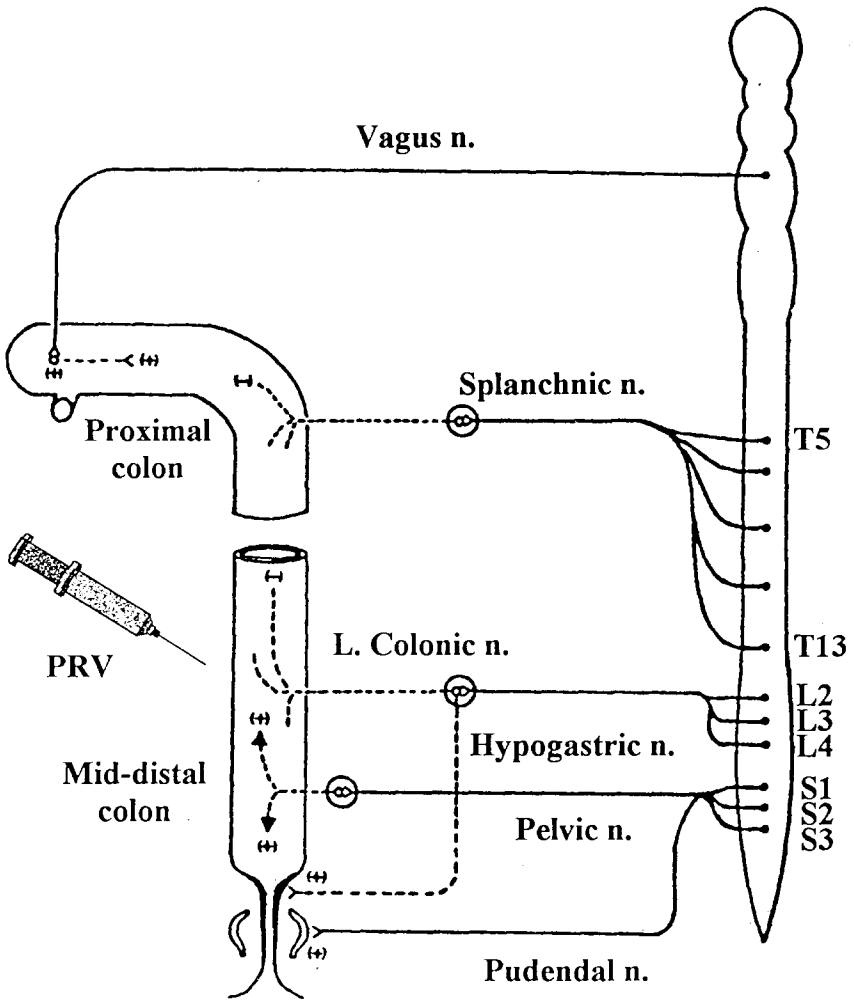

Vagus n.
Splanchnic n.
Proximal colon
T5
PRV
L. Colonic n.
T13
L2
L3
L4
Hypogastric n.
Mid-distal colon
S1
S2
S3
Pelvic n.
Pudendal n.

Figure 1

PRV into Colon (Parasympathetics)

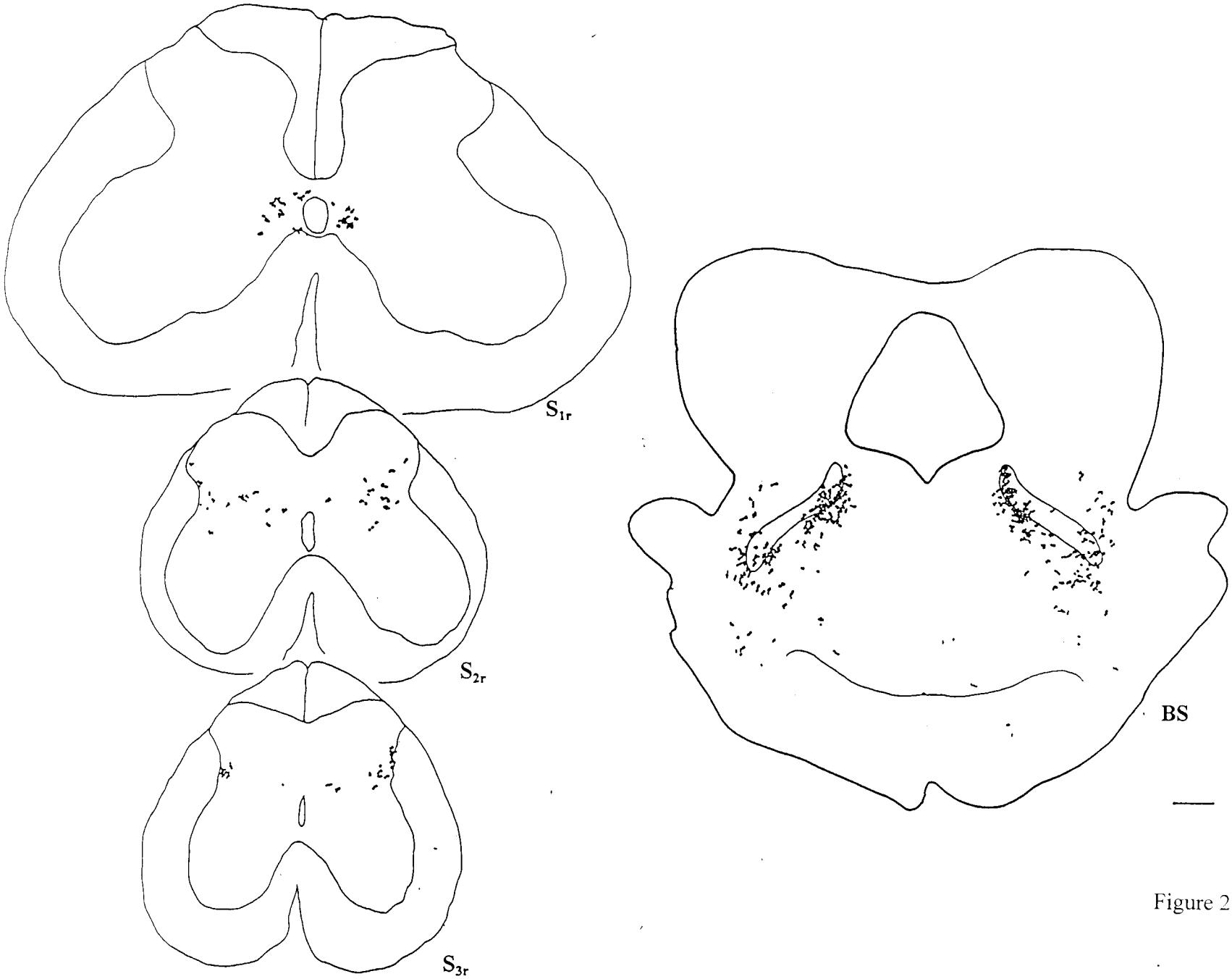


Figure 2

PRV into Colon
(Sympathetics)

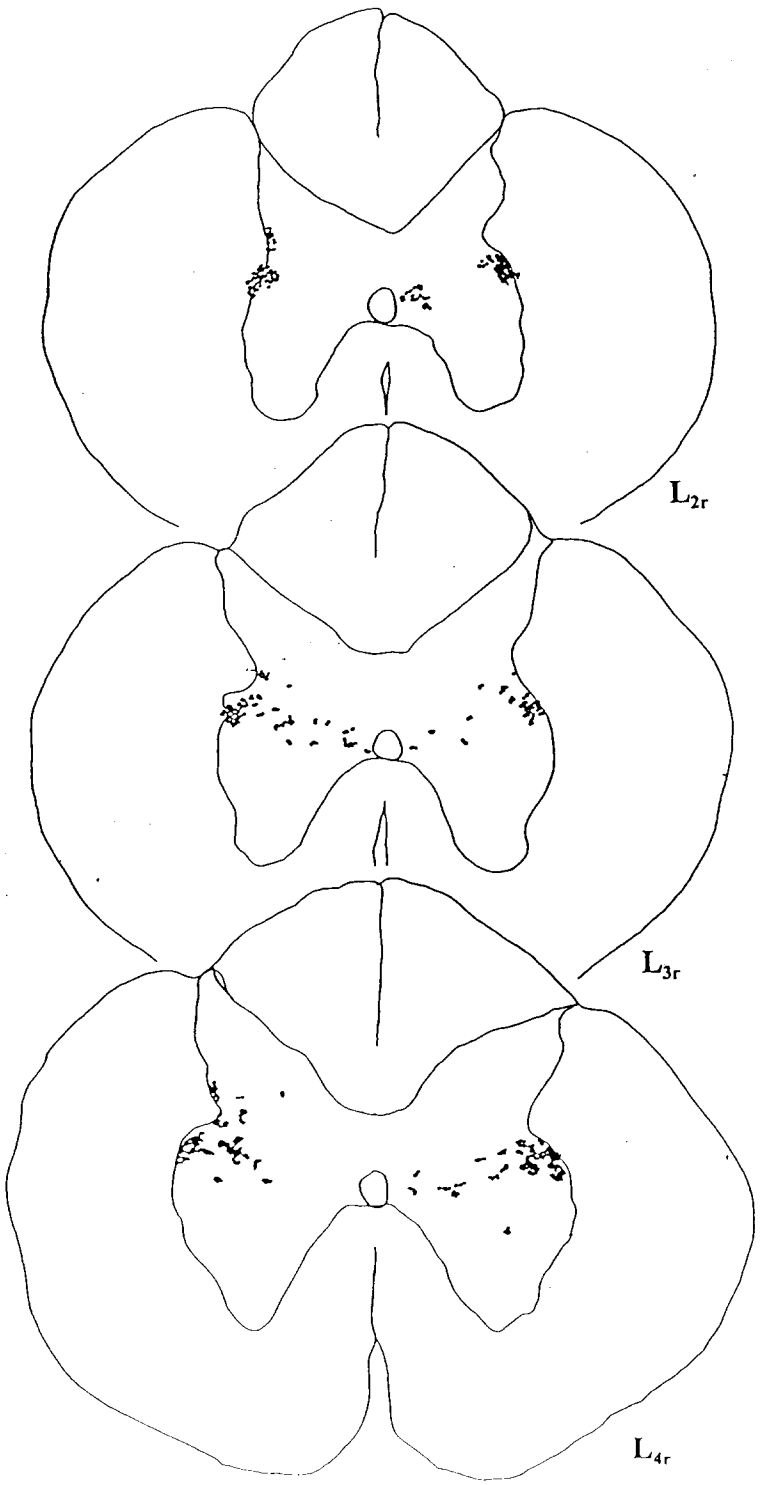


Figure 3

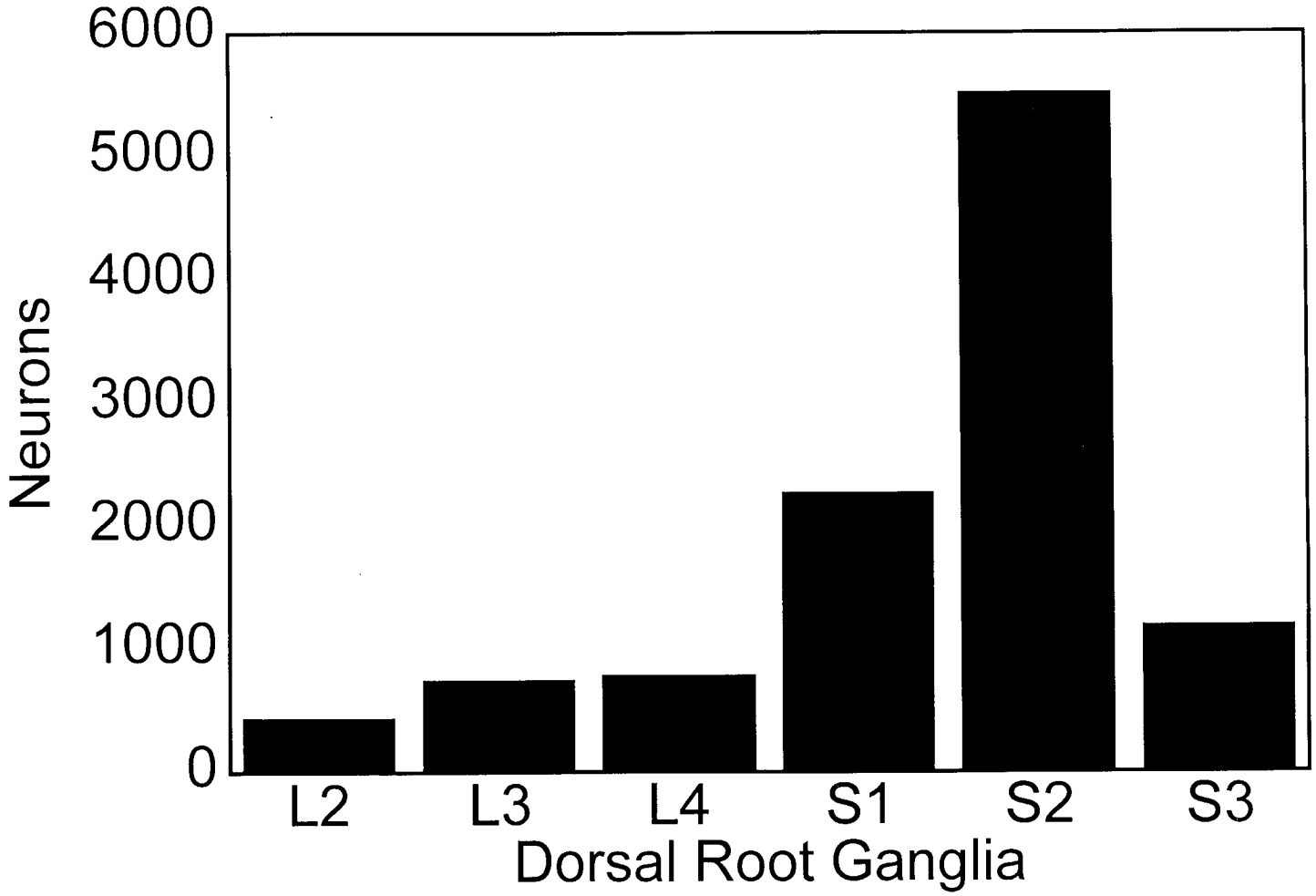
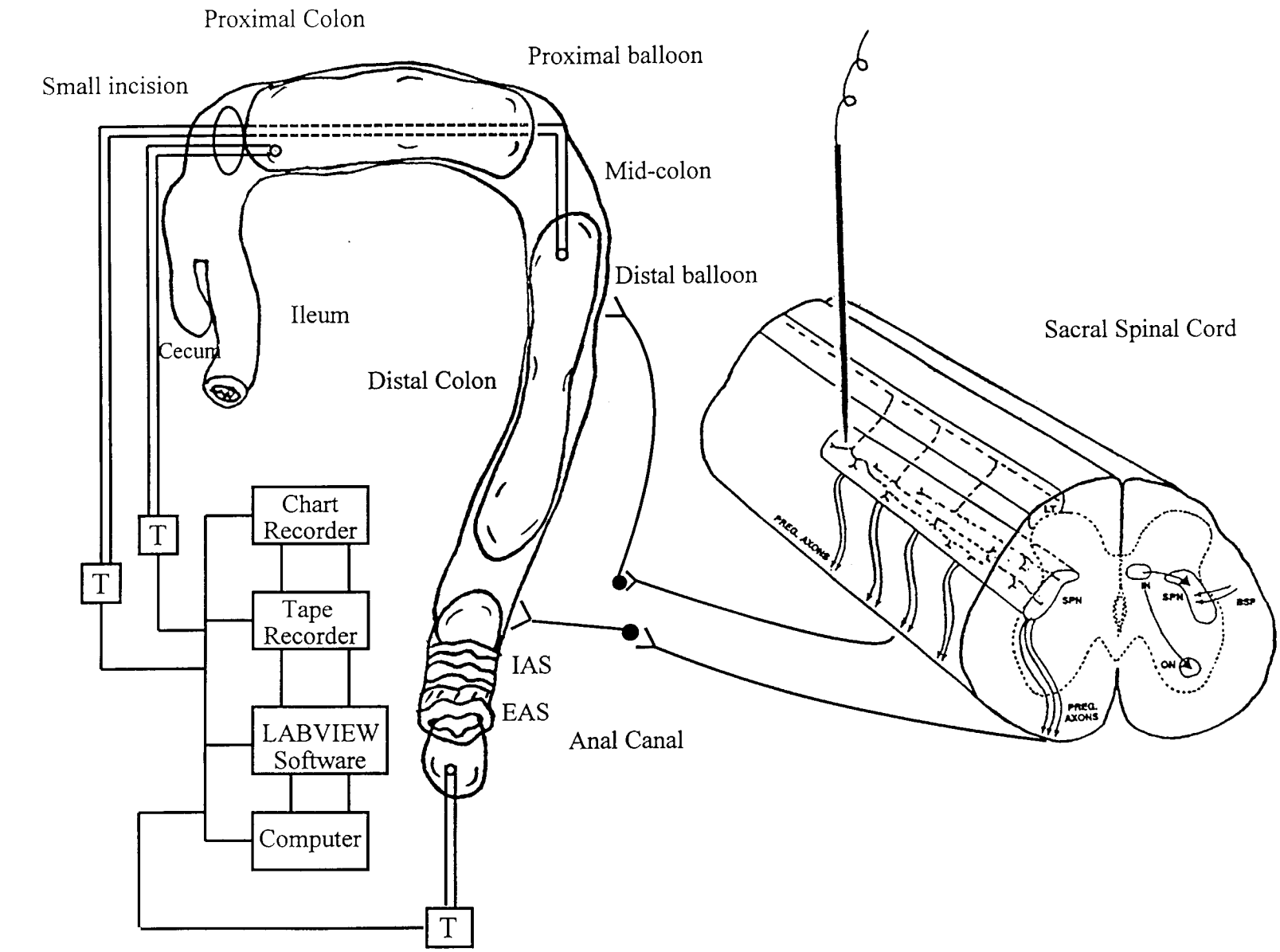

PRV Labeled Afferent Neurons
6000
5000
4000
3000
2000
1000
0
Neurons
L2
L3
L4
S1
S2
S3
Dorsal Root Ganglia

Figure 4



T = transducer

Figure 5

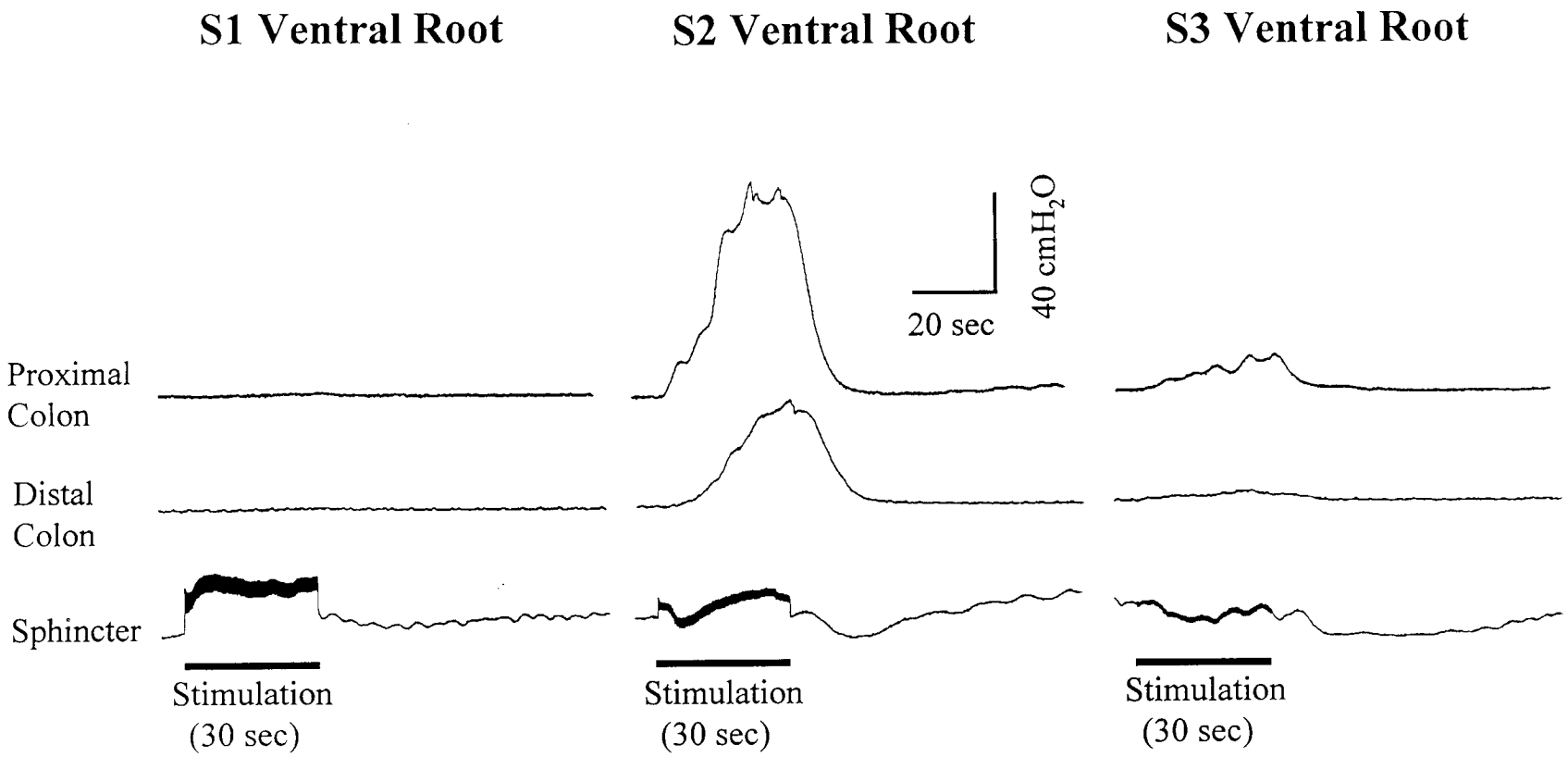

S1 Ventral Root
S2 Ventral Root
S3 Ventral Root
20 sec
40 cmH2O
Proximal Colon
Distal Colon
Sphincter
Stimulation (30 sec)
Stimulation (30 sec)
Stimulation (30 sec)

Figure 6

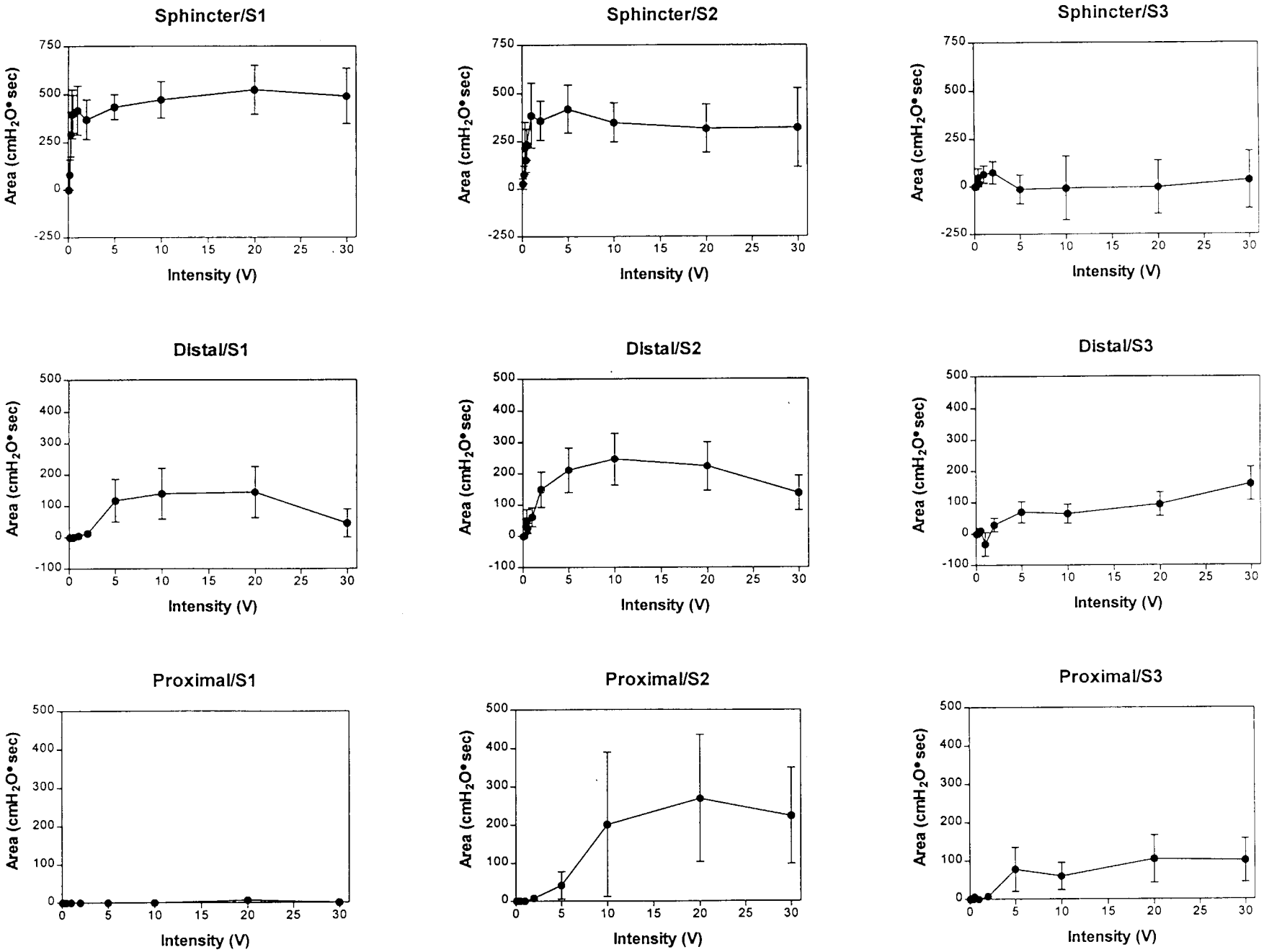
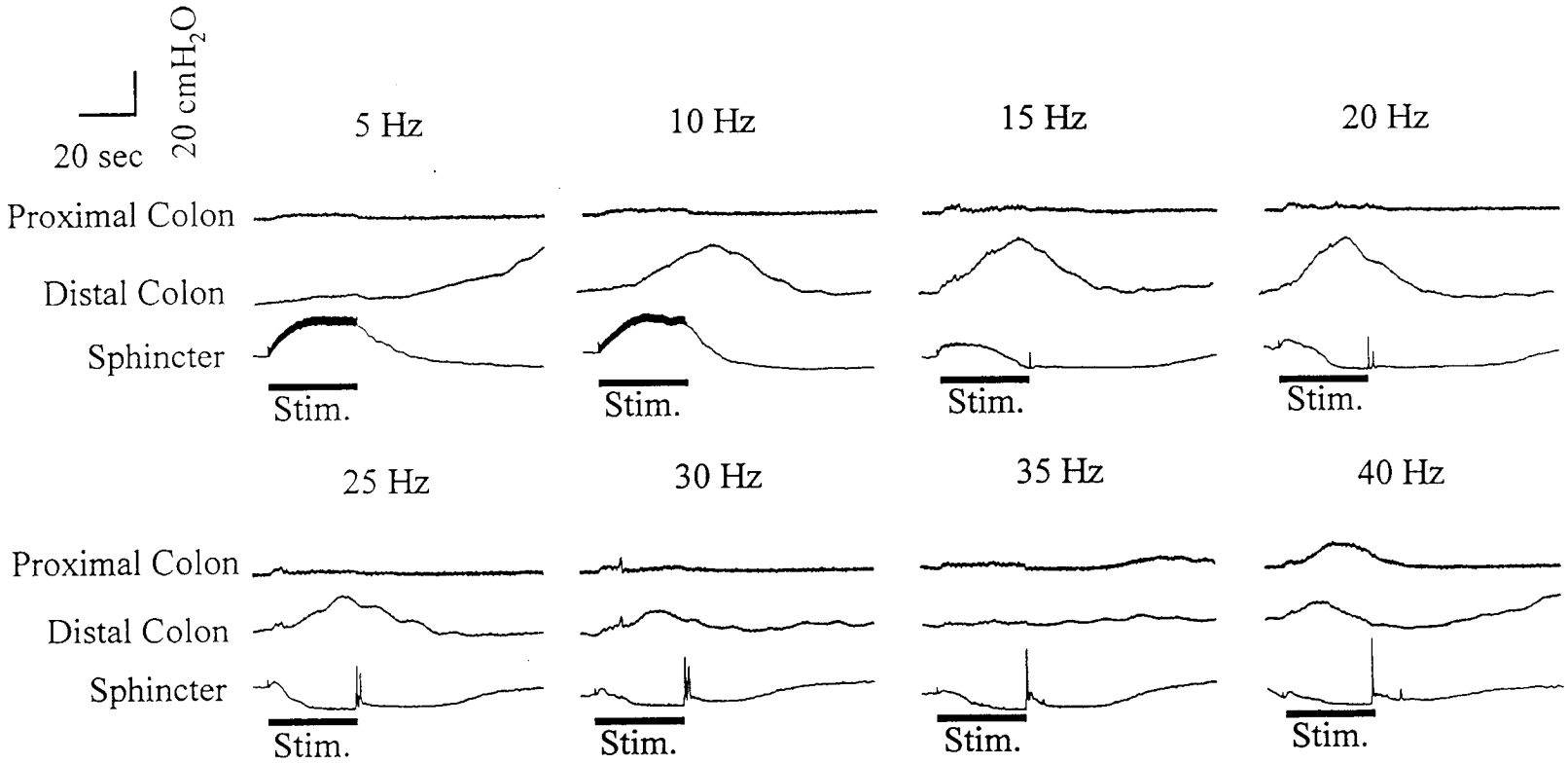
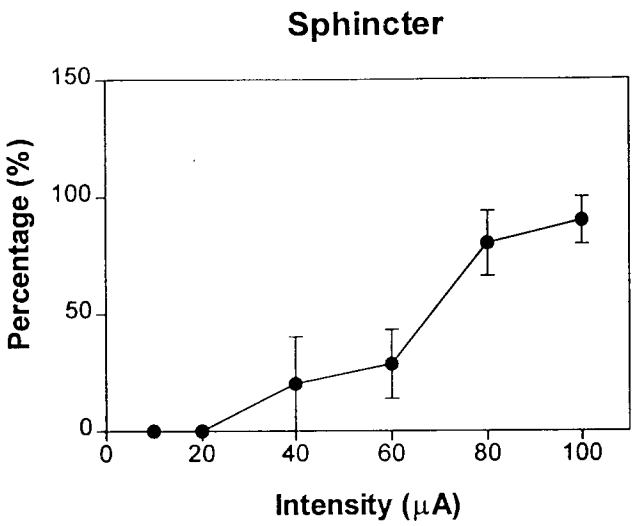

Sphincter/S1
Sphincter/S2
Sphincter/S3
Distal/S1
Distal/S2
Distal/S3
Proximal/S1
Proximal/S2
Proximal/S3
Area (cmH2O*sec)
Intensity (V)

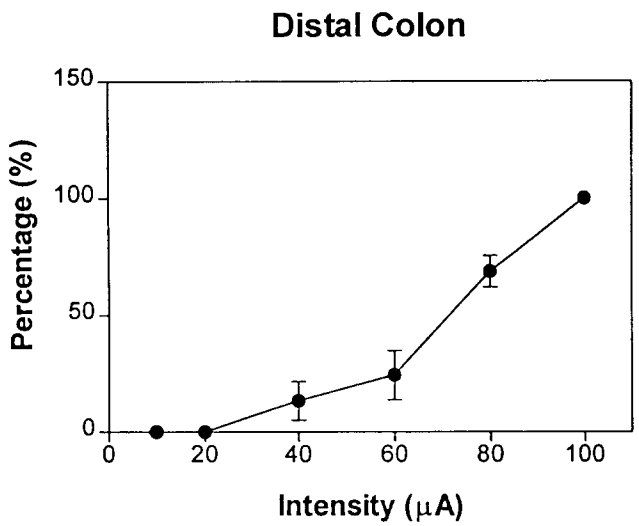
Figure 7



Stimulation: 0.2 ms pulse width, 100 μA intensity and 30 sec stimulation duration.
Data from MS#71, No.148,150,152,154-156,158 and 159. S2, Tract#3 at 1.2 mm depth.

Figure 8


Sphincter
150
100
50
0
Percentage (%)
0
20
40
60
80
100
Intensity (μA)


Distal Colon
150
100
50
0
Percentage (%)
0
20
40
60
80
100
Intensity (μA)

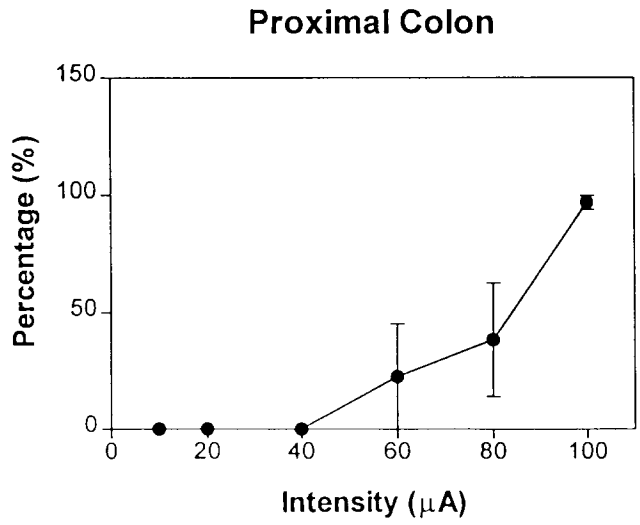
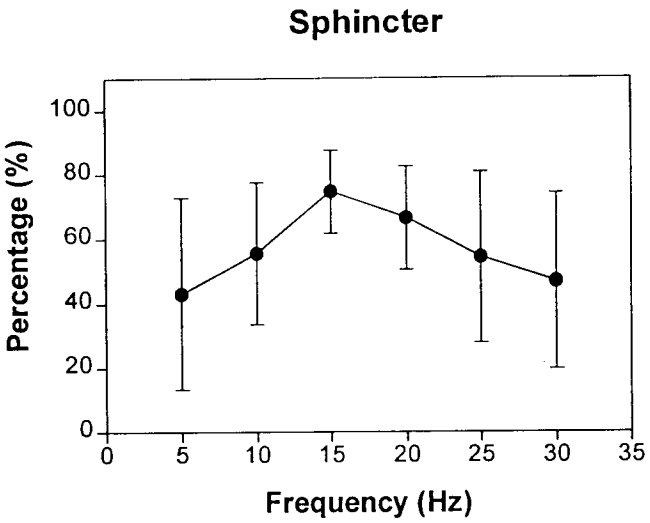
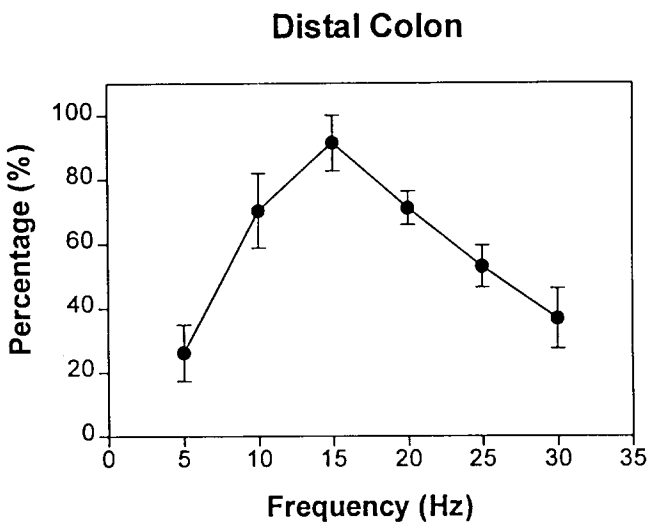

Proximal Colon
150
100
50
0
Percentage (%)
0
20
40
60
80
100
Intensity (μA)

Figure 9


Sphincter
Percentage (%)
100
80
60
40
20
0
0
5
10
15
20
25
30
35
Frequency (Hz)


Distal Colon
Percentage (%)
100
80
60
40
20
0
0
5
10
15
20
25
30
35
Frequency (Hz)

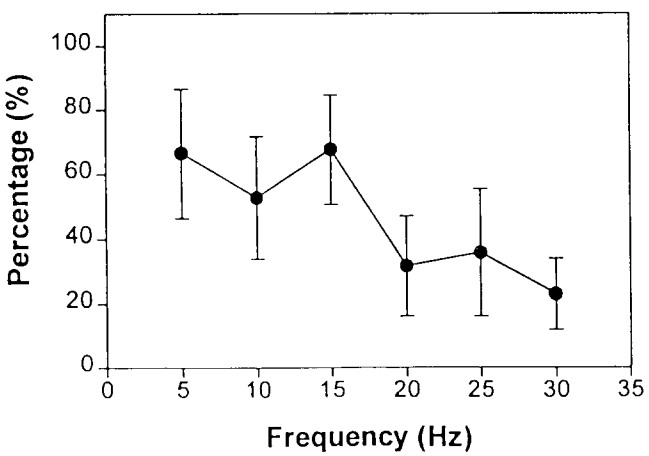
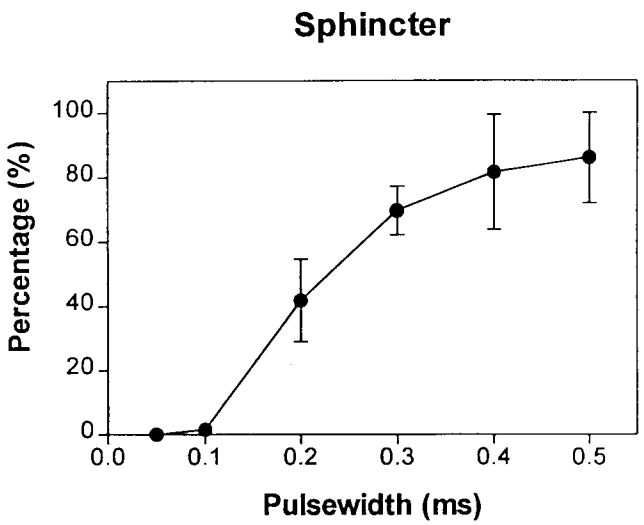
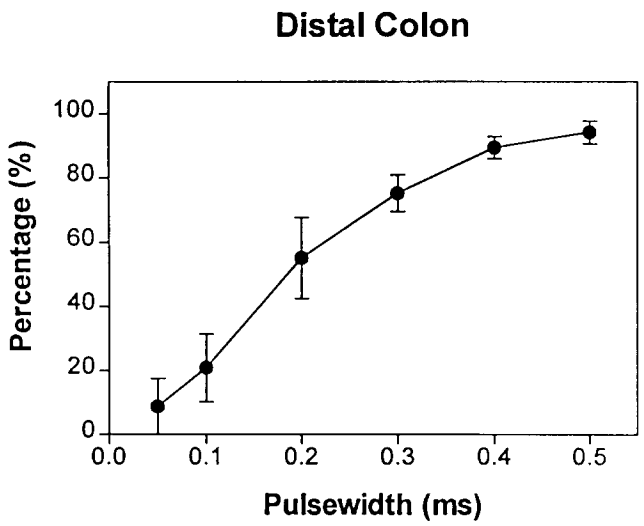

Proximal Colon
Percentage (%)
100
80
60
40
20
0
0
5
10
15
20
25
30
35
Frequency (Hz)

Figure 10


Sphincter
Percentage (%)
100
80
60
40
20
0
0.0
0.1
0.2
0.3
0.4
0.5
Pulsewidth (ms)


Distal Colon
Percentage (%)
100
80
60
40
20
0
0.0
0.1
0.2
0.3
0.4
0.5
Pulsewidth (ms)

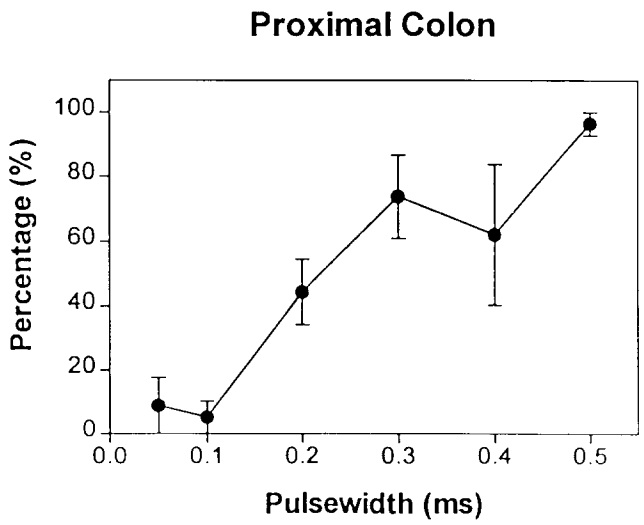

Proximal Colon
Percentage (%)
100
80
60
40
20
0
0.0
0.1
0.2
0.3
0.4
0.5
Pulsewidth (ms)

Figure 11

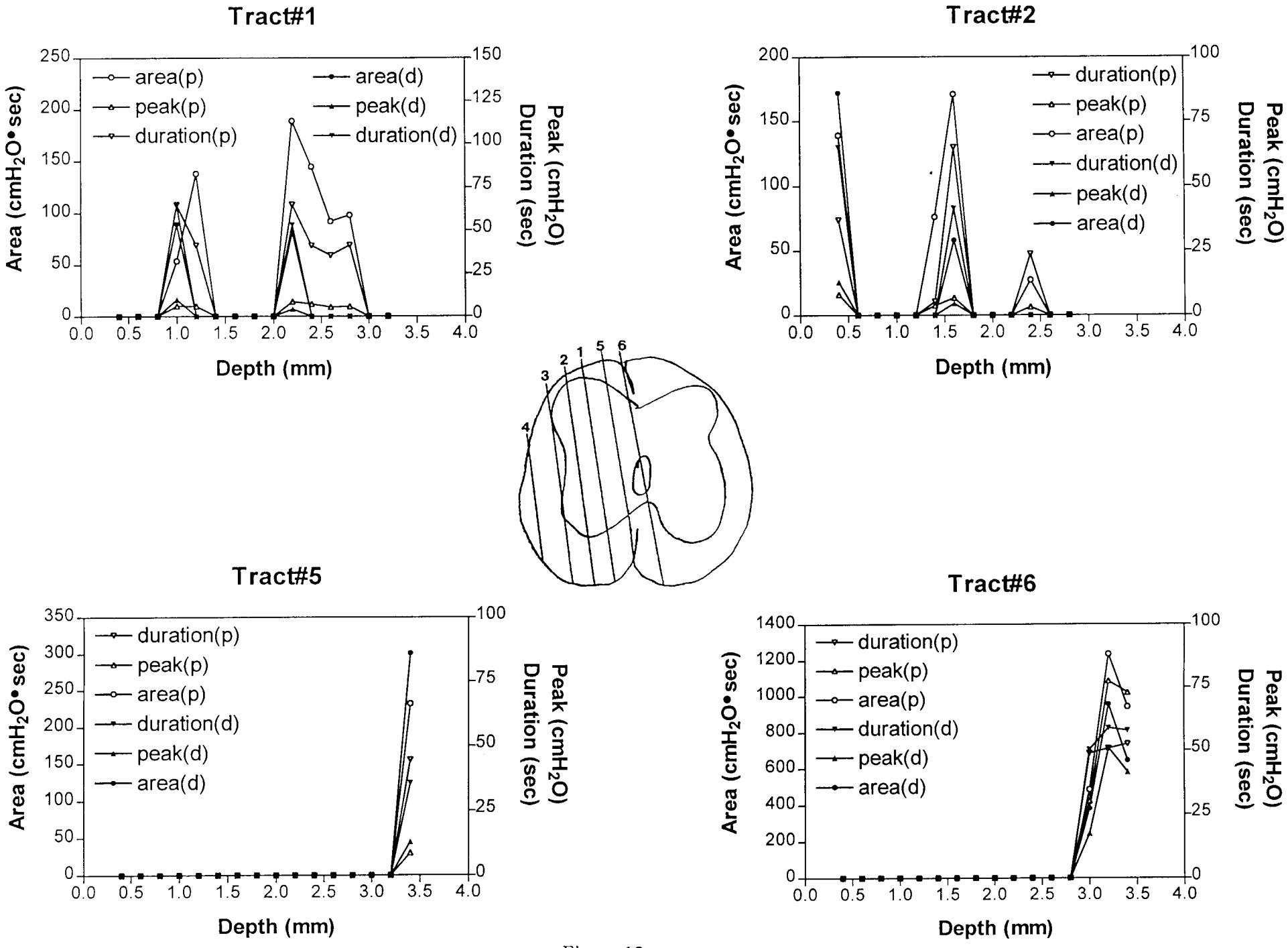

Tract#1
Area (cmH2O•sec)
Peak (cmH2O)
Duration (sec)
area(p)
peak(p)
duration(p)
area(d)
peak(d)
duration(d)
Depth (mm)
Tract#2
Area (cmH2O•sec)
Peak (cmH2O)
Duration (sec)
duration(p)
peak(p)
area(p)
duration(d)
peak(d)
area(d)
Depth (mm)
1
2
3
4
5
6
Tract#5
Area (cmH2O•sec)
Peak (cmH2O)
Duration (sec)
duration(p)
peak(p)
area(p)
duration(d)
peak(d)
area(d)
Depth (mm)
Tract#6
Area (cmH2O•sec)
Peak (cmH2O)
Duration (sec)
duration(p)
peak(p)
area(p)
duration(d)
peak(d)
area(d)
Depth (mm)

Figure 12